

Effect of foliar fertilization of microelements on highbush blueberry (*Vaccinium corymbosum* L.) nutrient status and yield components in cutover peatlands

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Abstract. The commercial cultivation of highbush blueberry in Latvia was successfully started during the last 20 years. In 2018, there was an estimated 280 ha of highbush blueberries planted in Latvia with increasing annual hectareage. In general, blueberry is a highly specialized crop that has definite soil agrochemical and climatic requirements: it has low nutrient needs and is sensitive to excessive nutrient levels in the soil. However, balanced and precise mineral nutrition is essential for producing high and quality yield. The study was conducted in the producing planting of highbush blueberry, cultivar ‘Patriot’, during the vegetation season of 2018. The research was carried out to determine the effect of foliar fertilization with micronutrients (Fe, Zn, Cu, Mo, B) on the productivity, the content of mineral elements in leaves and photosynthesis of blueberries. Field experiment design included foliar fertilizer treatments 0 to 3 times per season. In general, our results revealed that foliar sprays with micronutrients had a positive effect on the berry yield, parameters of photosynthesis and microelement content in leaves of highbush blueberry. It was determined that the application of the foliar fertilizer 4 times per season provided the highest berry yield (134% compared to control) and the highest photosynthetic activity of plants. Our study suggests that correct foliar fertilization can optimize the content of Fe, Zn and B in blueberry leaves.

Key words: field experiment, soil nutrient status, leaf nutrient status, parameters of chlorophyll fluorescence.

INTRODUCTION

Highbush blueberry (*Vaccinium corymbosum* L.) is a perennial flowering plant from the family *Ericaceae* of the genus *Vaccinium*. Commercial blueberry varieties, in general, are indigenous to eastern and central North America including the eastern territories of Canada (Trehane, 2004). Commercial production of *Vaccinium* species in the United States of America has existed since the latter part of the eighteenth century (Eck, 1990). Today, highbush blueberries are cultivated in many non-native regions worldwide in highly different soil and climatic conditions (Strik, 2005). Many species of *Vaccinium* have a long history of being used for medical purposes. Blueberries are also valued for their fresh taste as well as their potential for being processed. Increasing demand for healthy ingredients from the food industry and changed consumer

consciousness provides a great opportunity for further progress of blueberry production. The cultivation of blueberries in Latvia is comparatively recent – while the first experimental plantations were established in the middle of 1980s, commercial cultivation of these berries started in last 20 years (Osvalde et al., 2018) reaching 280 ha in 2018 (Karlsons et al., 2018). In general, about 85% of the global production of blueberries comes from the United States of America and Canada alone (Crop statistics, 2019).

There are two different soil types used for the cultivation of highbush blueberries in Latvia: light acid mineral soils rich in organic matter and peat soils. Today, about one-third of the total hectareage of blueberry plantings in Latvia are successfully developed on cutover peatlands on bare sphagnum peat (Osvalde et al., 2018).

Although highbush blueberries have specific soil requirements and are well adapted to low pH soils with limited availability of mineral elements, balanced mineral nutrition is vitally essential in producing high and qualitative yield (Hart et al., 2006; Pormale et al., 2009). Considering that blueberry plants are shallow-rooted, fruit production can be significantly reduced even with a moderate nutrient deficiency. On the other hand, excessive or inadequate fertilization is potentially damaging to blueberry cultivation especially in plantations established in environmentally sensitive areas such as excavated peat bogs. Especially considering that blueberry fruit value is relatively high; it contributes to the tendency to apply more types or quantity of fertilizers with the hope of improving yield or quality. Results from the previous studies in 2006–2017 highlighted the incompleteness in highbush blueberry providing with nutrients that could be a significant restrictive factor for obtaining high, qualitative and sustainable berry yields in Latvia. Overall, more than 50% of blueberry soils had a low content of N, S, Mo and B (Osvalde et al., 2018). Considering that average yield in Latvia (1.49 t ha^{-1}) is significantly lower to compare with the United States of America (6.91 t ha^{-1}), Canada (3.28 t ha^{-1}) and Poland (3.07 t ha^{-1}) (Crop statistics, 2019) research on mineral nutrition as one of the potential limiting factors of reduced yield of blueberry in Latvia are critically important. Plant fertilizers can be applied directly to the soil for uptake by plant roots, by foliar spraying for uptake via the leaves, or in combination. Availability of nutrients from the soil may be limited in the conditions of improper soil reaction, ionic antagonism, or unfavorable weather conditions. Under such circumstances, foliar sprays are a simple, fast and effective method for supplying nutrients to plants (Fageria et al., 2009; Wach & Błażewicz-Woźniak, 2012). Main advantages of foliar fertilization: it is a possibility to react rapidly to visual symptoms or tissue analysis, fast plant response in correcting the deficiency, avoidance of soil problems, relatively low cost, small amounts of fertilizer, and reduced risk of environmental pollution. Therefore, used wisely, foliar fertilizers may be more environmentally friendly and target oriented than soil fertilization though plant responses to foliar sprays are variable and many of the principles of foliar fertilization remain poorly understood (Fernández et al, 2013).

The research was carried out to determine the effect of foliar fertilization with micronutrients (Fe, Zn, Cu, Mo, B) on the productivity, yield quality, leaf nutrient status and photosynthesis of highbush blueberries.

MATERIALS AND METHODS

Field experiment

The field experiment was carried out in 2018 by the Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia, on a production farm (56°70'N, 23°60'E) established on a excavated peat bog abandoned after industrial peat production (region of Jelgava) to investigate the effect of micronutrient foliar application on blueberry yield and berry quality. Together 128 (10 years old) blueberry plants (32 for each treatment) of 'Patriot' cultivar was used in the spacing of 1.2×2.0 m (3,888 plants ha⁻¹). The experiment followed a randomized sub-block design. Peat chemical characteristics, determined from composite peat sample of the upper 20 cm taken in April, before the start of the experiment are given in Table 1. In general, peat from the experimental field was characterized by low levels of N, S, Mo, B and consequently low pH_{KCl} (4.31), as well as high organic matter content (< 95%). At the beginning of May (beginning of the active growing period for plants in Latvia climate conditions), the complex fertilizer suitable for blueberries: 12–8–16 + microelements (Blaukorn classic, COMPO EXPERT GmbH, Germany) was applied at a dose of (according to the producer's instructions) 400 kg ha⁻¹ for all treatments. Within the growing period, the bushes were sprayed with foliar fertilizer containing: Fe, Zn, Cu, Mo, B (Yara Vita, Yara International ASA) as follows:

1. Control – 0 treatments per season.
2. Treatment 1 – 1x per season (01.06. 2018).
3. Treatment 2 – 2x per season (01.06. 2018; 05.07.2018).
4. Treatment 3 – 3x per season (01.06. 2018; 05.07.2018; 03.08.2018).

The blueberry leaves for chemical analysis were collected and fluorescence parameters measured 10 days after each foliar fertilizer treatment. Berry yield, as well as the yield components, were determined for each treatment at a harvest time. Berries were harvested twice (21.07.2018 and 10.08.2018) by hand-picking, in the first harvest, all mature fruits were harvested, the second time all mature and unripe fruits were harvested, the total yield was measured for all berries, but average fruit diameter and one berry mass only for mature berries.

Fluorescence parameters were measured using a Handy PEA chlorophyll fluorometer on field conditions. Fluorescence measurements were performed according to the manufacturer's instructions as described previously (Samsone et al., 2009). Before analysis, leaves were dark adapted for 30 min using the appropriate leaf clips. For every treatment, 15 independent measurements on individual leaves were performed. Performance Index (PI) and potential maximum quantum yield of photosystem II (FV/FM) were determined. The data were analyzed by PEA Plus software.

Laboratory analysis and measurements

Soil samples were separately taken at 0 to 20 cm depth from each treatment. Each soil sample (2 L) consisted of thoroughly mixed five subsamples. To determine nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) concentration the air-dried and 2 mm-sieved soil samples were extracted using 1 M HCl (soil-extractant mixture 1:5 v/v). Oxidation of soil extract with conc. HNO₃, H₂O₂ and HClO₄ were performed for determination of P, S, and Mo. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated

by atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, acetylene-air flame) (Anonymous, 2000) those of N, P, Mo, B by colorimetry, S by turbidimetry, and K by a flame photometer (Jenway PFP7, air propane-butane flame). All spectroscopic, colorimetric or photometric determinations were performed in triplicates. For soil, the concentrations of all mineral elements were given as mg L^{-1} .

Soil electrical conductivity (EC) was measured in distilled water extraction (soil – distilled water mixture 1:5) with the conductometer Hanna EC 215, but the soil reaction was detected in 1 M KCl extraction (soil-extractant mixture 1:2.5) using the pH-meter Sartorius PB-20.

For each plant sample 100–200 blueberry leaves were collected. The leaf material was oven-dried at 60 °C to a constant weight and finely ground using a laboratory mill. Then the samples were dry-ashed in concentrated HNO_3 vapors and re-dissolved in HCl solution (HCl – distilled water mixture 3:100) (Rinkis et al., 1987). Concentrations of 5 microelements (Fe, Zn, Cu, Mo, B) were determined in all leaf samples. Nutrients were analyzed using the same procedures as in the case of soil samples. Microelement concentrations in plant tissue were expressed as mg kg^{-1} .

All chemical analyses of soil and plant samples were done in the Laboratory of plant mineral nutrition of the Institute of Biology, University of Latvia.

Statistical analysis

The levels of statistical significance were determined with MS Excel 2016. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis. The Student's t-test (Two-Sample Assuming Equal Variances) was used for testing the differences between treatments.

RESULTS AND DISCUSSION

To characterize the soil nutrient status in a planting of highbush blueberry before and during the experiment, the plant available concentration of 12 essential nutrients, pH_{KCl} and EC levels were estimated in peat samples and evaluated in relation to the guideline values (Table 1).

Chemical analyses of peat before experiment establishment confirmed significant deficiency of N (9 mg L^{-1}) and S (10 mg L^{-1}) in the experimental field, as well as low level or deficiency of K, Cu, B and Mo in comparison to optimal values reported by Nollendorfs (2004) for blueberries: N – $70\text{--}150 \text{ mg L}^{-1}$, S – $40\text{--}80 \text{ mg L}^{-1}$; K – $80\text{--}120 \text{ mg L}^{-1}$; Cu – $4\text{--}8 \text{ mg L}^{-1}$; B – $0.6\text{--}1.2$; Mo – $0.10\text{--}0.25 \text{ mg L}^{-1}$. Previously frequent deficiency of N and S in soil tests for blueberry plantations in Latvia established in peat soil was reported by Osvalde et al. (2018). As nitrogen is one of a key element in blueberry nutrition, adequate fertilization is necessary to maintain renewal growth, crop production, and flower bud development for next year's crop. Numerous studies have been proved the importance of proper N fertilization for successful blueberry growing (Bañados et al., 2012; Bryla et al., 2012; Ehret et al., 2014). However, fertilization with higher-than-recommended N rates could lead to significant yield decreases over time and risk of environmental contamination (Messiga et al., 2018). Our results demonstrated that a granular complex fertilizer applied in spring was sufficient to maintain appropriate N content in peat up to October. Though N concentrations were slightly less than recommended, it should be taken into account that a sustainable N management strategy

requires the lower amounts of nitrogen for mature blueberries (Throop & Hanson, 1997; Bryla & Machado, 2011). In general, applied complex fertilizer provided a sufficient level of all macro- and microelements during the vegetation season in the experimental field, with the exception of Mo. Peat chemical analyzes suggested that established pH values (4.31–4.64) corresponded to the guidelines recommended by several authors for blueberries (Nollendorfs, 2004; Hart et al., 2006; Paal et al., 2011).

Table 1. Nutrient concentrations in 1 M HCl extraction (mg L⁻¹) in highbush blueberry peat soil (Soil standards developed by Nollendorfs (2004))

Variable	April (30.04.2018)	July (05.07.2018)	August (03.08.2018)	October (05.10.2018)	Optimal for highbush blueberries in peat soils		
N	9 ± 0.75	64 ± 6.3	49 ± 3.0	47 ± 4.2	70	-	150
P	99 ± 13.00	212 ± 33.26	168 ± 21.89	149 ± 14.32	50	-	90
K	64 ± 5.60	130 ± 15.23	117 ± 6.36	94 ± 7.42	80	-	120
Ca	2,050 ± 298.61	3,100 ± 363.32	3,300 ± 198.36	3,350 ± 302.01	500	-	1,000
Mg	276 ± 30.31	350 ± 22.36	370 ± 18.33	500 ± 32.69	100	-	180
S	10 ± 1.19	90 ± 9.56	83 ± 5.36	75 ± 2.69	40	-	80
Fe	103 ± 7.05	170 ± 9.87	203 ± 8.69	130 ± 3.23	60	-	150
Mn	4.91 ± 0.79	9.0 ± 0.63	10.0 ± 1.10	8.0 ± 0.58	3.0	-	6.0
Zn	4.95 ± 0.58	10 ± 0.66	9.5 ± 0.45	6 ± 0.36	4.0	-	8.0
Cu	1.51 ± 1.16	8.2 ± 0.53	13.5 ± 1.32	8.5 ± 0.69	4.0	-	8.0
Mo	0.02 ± 0.003	0.03 ± 0.003	0.03 ± 0.006	0.04 ± 0.003	0.1	-	0.2
B	0.13 ± 0.02	1.00 ± 0.08	0.60 ± 0.04	0.5 ± 0.09	0.6	-	1.2
pH _{KCl}	4.31 ± 0.17	4.56 ± 0.23	4.64 ± 0.17	4.54 ± 0.32	4.5	-	4.8
EC (mS cm ⁻¹)	0.40 ± 0.03	1.66 ± 0.23	1.21 ± 0.20	1.31 ± 0.32	0.8	-	1.2

The chemical composition of leaves

It is well known that climatic conditions and various environmental factors such as relative humidity and temperature, have an effect on the properties of cuticular membrane, on the physiological processes of plants and on the properties of the fertilizer solution, and thus play an significant role in the process of absorption of mineral components by the cells of the leaf epidermis (Wójcik, 1998; Wach & Błażewicz-Woźniak, 2012). Overall, the research results from field experiment demonstrated a significant influence of the applied foliar fertilizer on the content of Fe, Zn and B in the highbush blueberry leaves. Only small impact or trend with no significant differences among treatments was established in the case of Cu and Mo, therefore, data are not presented.

Severe Fe deficiency (40–46 mg kg⁻¹) in blueberry leaves, collected prior to the use of foliar micronutrients, were detected at all experimental plots. According to current nutrient standards (Nollendorfs, 2004; Hart et al., 2006) the lower limit of the sufficiency range is 60 mg kg⁻¹ (Fig. 1, A).

After the first spray, the Fe concentration in the leaves exceeded the minimum sufficiency level, but after the second treatment with foliar fertilizer reached the optimum range. In general, foliar fertilization provided from 52% (after one spraying) to 118% (after 3 sprayings) higher Fe content in leaves compared to the control group. It should be noted that despite the Fe deficiency in the leaves in the spring, Fe

concentration in soils (103–203 mg L⁻¹) was optimal throughout the season. Such phenomenon could be explained by many biotic and abiotic factors influencing the availability of nutrients for plant uptake (Marshner, 2012) and underline the potential of foliar fertilization and importance of complex diagnostics (soil + plant tissue analysis) not only for correct determination of the nutritional status of plants but also for selection the most efficient mode of fertilizer application.

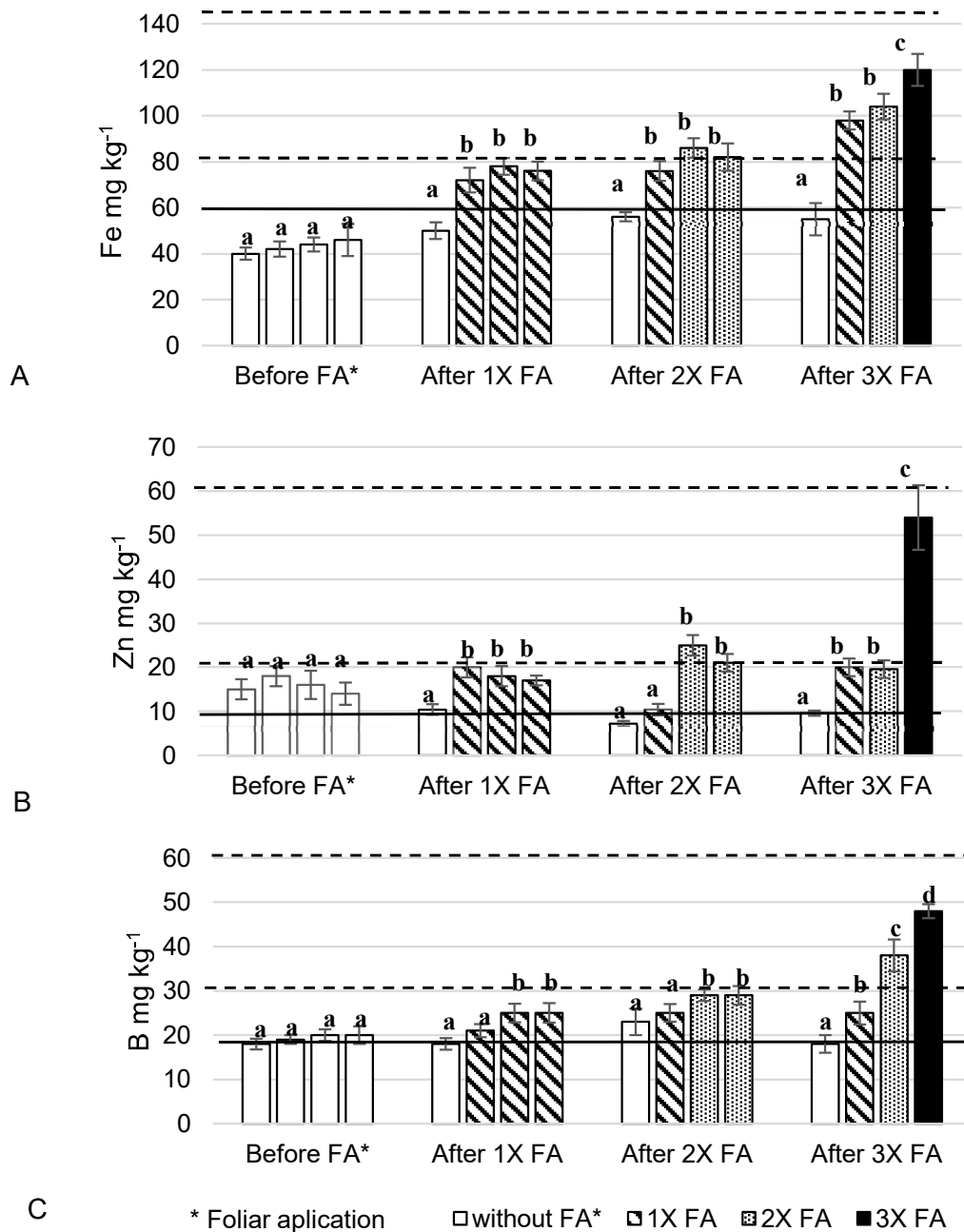


Figure 1. Concentration of Fe (A), Zn (B) and B (C) in highbush blueberry leaves. Means with different letters for each sampling time were significantly different (t-Test, $p < 0.05$). Minimum level ——— optimum range - - - - -.

Micronutrient Zn and B content in blueberry leaves were significantly affected by micronutrient foliar application (Fig. 1, B & C). It should be noted that sufficient Zn concentration level in soil (Table 1.) did not provide optimal content of this element in blueberry leaves at the beginning of the vegetation season. As mentioned before such phenomenon is caused by limited availability of particular mineral element for uptake in plants. As the B concentration in the soil was low at the beginning of the experiment, it was not surprising that the content of B in the leaves also did not reach the optimal level in spring. In general, results indicated that micronutrient foliar application provided/ensured Zn and B absorption in plants and increased the concentrations of these nutrients in blueberry leaves. Similarly, as in the case with Fe, the highest Zn and B concentration in leaves were recorded when plants were treated 3 times per season with foliar fertilizer. Researches made by Wójcik (2005) and Arrington & De Vetter (2017) claims that the application of foliar B fertilization resulted in a significant increase of B in highbush blueberry leaves, but not resulted in higher yield of berries. Similarly, Chen et al. (1998) detected no significant differences in fruit set and yield between B treatments in 12 lowbush blueberry (*Vaccinium angustifolium* Ait.) clones. In contrast, Meriño-Gergichevich et al., (2016) found that foliar B application not only elevated B concentration in plant leaves but significantly enhanced highbush blueberry yield and quality. Considering that boron is easily leached from soils, several studies (Gupta et al., 1985; Eaton & Sanderson, 2007) suggests that foliar applications of B as a supplement for soil fertilization method are a valuable tool to enhance boron supply in blueberry leaves.

Our research clearly indicates that foliar fertilization may be effective to ensure optimal crop performance in cases when a particular mineral element is not available to plants. Since small quantities of fertilizers are used by spraying directly to the bushes, this method could contribute also to environmental protection.

Chlorophyll fluorescence

Chlorophyll a fluorescence measurements were used as an indicator to analyze the effect of foliar fertilization on the physiological state of blueberries. Within the last decades, chlorophyll a fluorescence technique has been employed in a wide range of studies in plant biology. It has been shown that the decline of the ratio of variable to maximum fluorescence or potential maximum quantum yield of the photosystem II (FV/FM) below 0.8 reflects inhibition of photosynthesis and, therefore, indicates episodes of previously suboptimal conditions (Öquist et al. 1992; Andersone et al., 2011). On the other hand, Performance Index (PI) is a more complex parameter reflecting overall efficiency of light absorption, as well as both light and dark redox reactions (Strauss et al., 2006). Although the overall positive linear correlation between PI and FV/FM for analyzed blueberry plants was apparent, dynamics of changes in the individual parameters were not identical, suggesting the different effect of environmental factors on various aspects of the photochemistry of photosynthesis. Such difference partly can be explained by different time scales of impact by changing environmental conditions on the photochemistry, where PI shows instant general responses of the photosynthetic system while FV/FM reflects longer-lasting response. It is well known that Fe has an essential function in the whole photosynthetic apparatus and iron deficiency significantly influence parameters characterizing leaf functions,

including gas exchange, water status and chlorophyll fluorescence (Abadia, 1992; Fernandez et al., 2008; Fageria, 2009). Our research demonstrates that PI and partly FV/FM value reflected the direct effect of increased microelement supply on blueberries. The lowest PI values (Fig. 2) were established in spring when Fe deficiency and suboptimal concentrations of Zn and B was evident for all treatments. In turn, the highest PI and FV/FM, above recommended 0.8, were detected after the third spraying of fertilizers. The potential maximum quantum yield of photosystem II (FV/FM) showed that it tends to be equal from May to June, with a significant decrease in July (Fig. 2, B). Such decrease of FV/FM below 0.77 in July indicated possible photoinhibition of photosynthesis in highbush blueberry. As pointed out by Baker (2008), FV/FM represents the maximum quantum yield of photosystem II, while under ambient light conditions effective quantum yield may be significantly lower. The present data show that photochemistry of photosynthesis of blueberry is strongly affected by local changes of environmental conditions. However, it is evident that mineral nutrition conditions are among the most important factors affecting the physiological status of highbush blueberry.

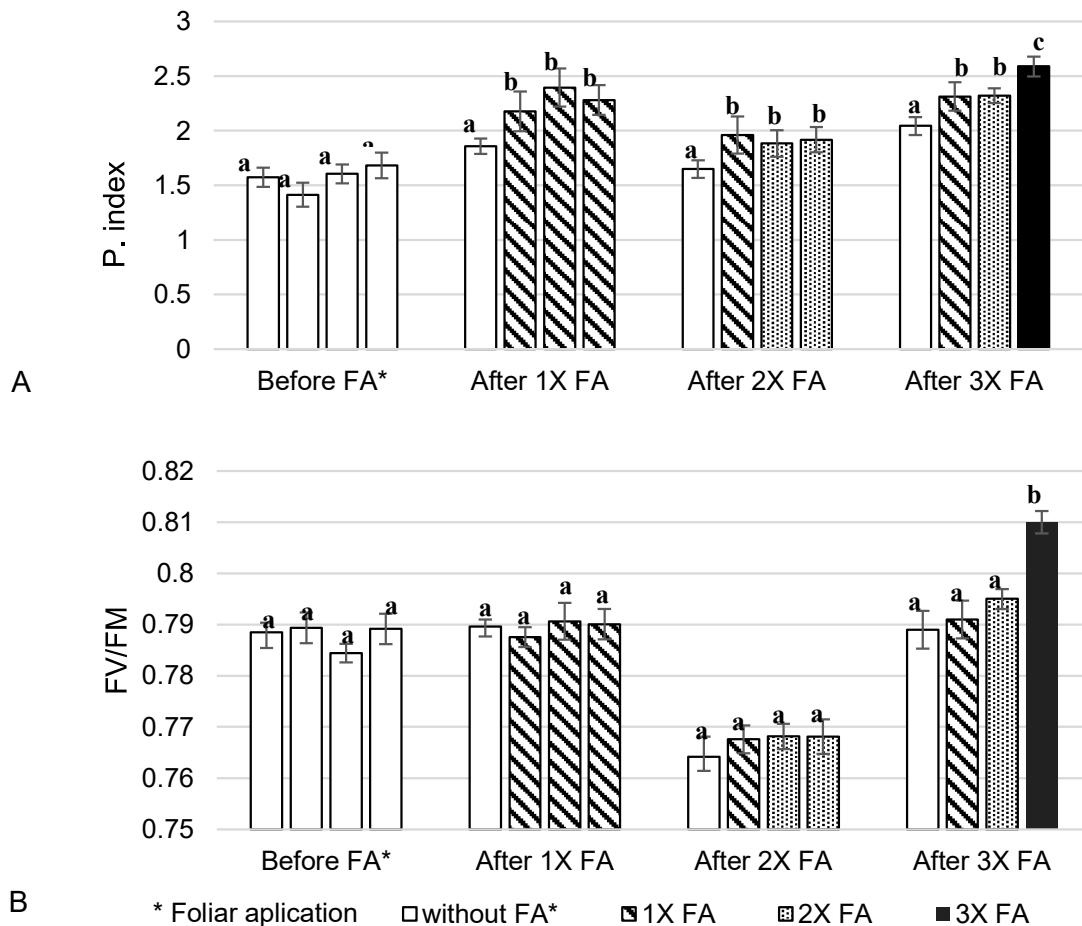


Figure 2. Changes of chlorophyll a fluorescence parameters PI (A) and FV/FM (B) in highbush blueberry leaves: PI – Performance Index; FV/FM – potential maximum quantum yield of photosystem II.

Berry yield and quality

The aim of the experiment was also to examine the blueberry yield and quality. In general, berry yield was significantly influenced by foliar fertilizer applications ($p < 0.05$). Our results showed that total blueberry yield (Table 2) achieved in the experiment was equivalent to 5.02–7.14 t ha⁻¹ (3,800 bushes per hectare) which are considerably higher compare to average blueberry yield in Latvia – 1.49 t ha⁻¹ (Crop statistics, 2019) and are close to average yields reached in USA (6.91 t ha⁻¹ in 2017) confirming that correct mineral nutrition is one of the most significant limiting factors for high berry fields in Latvia. The highest yield was obtained from the bushes sprayed 3 times per season. Well-known that fruit size is of decisive importance for the crop quality. In our experiment, the largest berries and the highest mass of one berry (23.0 mm and 3.36 g accordingly) were also achieved from the bushes sprayed 3 times per season. On the other hand, the smallest berries and the lowest one fruit mass were detected in control treatment. Our results are consistent with Starast et al. (2002) who obtained a higher yield and higher weight of fruit of lowbush blueberry under the effect of foliar fertilization in Estonia.

Table 2. Characteristics of the highbush blueberry ‘Patriot’ fruits in dependence on fertilizing

Treatment	Average yield from bush (kg)	Average one fruit mass (g)	Average fruit diameter (mm)
Control	1.32 ± 0.031a*	2.90 ± 0.322a	19.5 ± 0.27a
Treatment 1	1.48 ± 0.028a	2.86 ± 0.431a	19.6 ± 0.21a
Treatment 2	1.50 ± 0.033b	2.99 ± 0.809a	22.3 ± 0.33b
Treatment 3	1.88 ± 0.042b	3.36 ± 0.286b	23.0 ± 0.56b

*Means with different letters in a column were significantly different (t-Test, $p < 0.05$).

CONCLUSION

As a conclusion, our study reveals, that foliar fertilization can become a very practical and effective method for increasing blueberry yields and berry quality. In general, foliar application of micronutrients resulted in the better provision of these elements in leaves, the better photosynthetic performance of plants as well as higher and more qualitative yield of blueberries. Based on the present study, it can be suggested that chlorophyll a fluorescence is a useful tool not only for non-destructive measurement of stress impact on the physiological status of highbush blueberry but also to search for the effect of any suboptimal conditions through the analysis of various aspects of the photochemistry of photosynthesis.

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